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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Composition

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COMPOSITION

This invention relates to a composition, to foodstuffs, food supplements or pharmaceutical compositions comprising the composition, to uses of the composition and to a process for producing the composition.

Pine needles are the leaves of plants of the Pinaceae family, including the genus *Pinus*. Certain types of pine needles are available in abundant supply and have been used for various purposes. Pine needle extracts have been described as being useful in specific beverages in JP 08107778 A and JP 07059538. Rice cakes containing pine needle extracts are described in JP 01218562 A.

A process for the extraction of taxol from pine needles is described in WO 94/15483.

High blood pressure (or hypertension) is known to be associated with many medical problems. High blood pressure directly increases the risk of coronary heart disease and stroke. High blood pressure most predominantly occurs in people over 35 years old but environmental and genetic factors and some medical conditions, such as diabetes mellitus, gout or kidney disease can lead to an increased risk of high blood pressure in people of all ages.

WO 98/28990 discloses a method of preparing food seasonings, food ingredients and food items using plant sterols and/or stanols together with raised levels of one or more of magnesium, calcium and potassium. Ingestion of the food is said to lead to a decrease in both cholesterol level and blood pressure.

There remains a need for active materials that can reduce high blood pressure, particularly naturally-occurring materials that can be used as food supplements or in foodstuffs.

US 6,329,000 discloses the use of certain pine needle extracts for treating various diseases including myocarditis, angina, arrhythmia, diabetes, senile dementia, relatively simple extraction process using water and alcohol as solvents.

Pine needles and their extracts may contain isocupressic acids. Isocupressic acids have been described as causing toxicity problems in beef cattle.

It has surprisingly been found that isocupressic acids can be removed from pine needle extracts to form an extract which still exhibits therapeutic activity (such as the ability to lower blood pressure).

According to the present invention, there is provided a composition which is obtainable as an extract from pine needles, having therapeutic activity and comprising isocupressic acid compounds in an amount of less than 0.01 wt% (by weight based on the total weight of the composition).

In another aspect, the invention provides a foodstuff (for example a dairy based food product), food supplement or pharmaceutical composition comprising a composition of the invention.

A further aspect of the invention is a method of improving one or more properties of a food product selected from hardness, texture, aeration, spreadability, oral properties, mouthfeel, flavour, colour, viscosity, ease of processing and health

properties, which comprises incorporating into the food product a composition comprising one or more organic compounds, said composition being obtainable as an extract from pine needles. The properties are improved compared to an otherwise identical food product that does not contain the material.

The invention also provides a composition, foodstuff, food supplement, pharmaceutical composition or dairy based food product of the invention for use to lower blood pressure in mammals, particularly in humans.

In yet another aspect, the invention provides the use of a material comprising one or more organic compounds, said material being obtainable as an extract from pine needles, for improving one or more properties of a food product selected from hardness, texture, aeration, spreadability, oral properties, mouthfeel, flavour, colour, viscosity, ease of processing and health properties. The properties are improved compared to an otherwise identical food product that does not contain the material.

A yet further aspect of the invention is a process for producing a composition of the invention, which comprises the following steps:

treating pine needles with a solvent selected from water, organic solvents and mixtures thereof (preferably water), preferably at an elevated temperature of from 40 °C to 110 °C, to form a first extract;

removing isocupressic acid compounds from the first extract by treatment with an ion exchange resin (preferably whilst the first extract is in aqueous solution, more preferably at an elevated temperature); and

optionally, filtering and concentrating the treated extract to obtain the composition as a powder or a concentrate. Preferably, prior to step (a), the pine needles are pretreated with a non-polar solvent (e.g, an alkane having from 4 to 10 carbon

atoms, such as hexane), more preferably at a temperature of from 40 °C to 90 °C. This pretreatment typically removes at least a part of the isocupressic acids.

The composition of the invention, and products comprising the composition is capable of lowering blood pressure in mammals, particularly in humans. Therefore, the invention also involves a method of lowering blood pressure which comprises the administration of a composition, foodstuff, food supplement, pharmaceutical composition or dairy based food product of the invention. The invention also involves the use of a composition, foodstuff, food supplement, pharmaceutical composition or dairy based food product of the invention in the manufacture of a composition for treating and/or preventing hypertension.

Compositions of the invention can be in the form of solids or liquids, including solutions, suspensions and dispersions. Preferably, the composition is in the form of a powder or an aqueous solution.

It is surprising that the composition of the invention retains therapeutic activity, even though it has been treated to the extent that it comprises isocupressic acid compounds in an amount of less than 0.01 wt%, because it has been found that isocupressic acid compounds have activity in lowering blood pressure. The term therapeutic activity in this context means usefulness in the treatment, inhibition or prevention of diseases or disorders. Diseases and disorders include, but are not limited to, high blood pressure (hypertension).

The composition of the invention preferably contains isocupressic acid compounds in an amount of less than 0.005 wt%, more preferably less than 0.003 wt%, even more preferably less than 0.002 wt% such as less than 0.001 wt%. The terms

"isocupressic acid compounds" and "isocupressic acids" are used synonymously herein and refer to isocupressic acid itself and preferably related diterpene acids found in pine needles and their extracts, such as imbricatoloic acid, agathic acid, dihydroagathic acid and tetrahydroagathic acid. Preferably, the composition is free of isocupressic acids or substantially free of isocupressic acids (i.e., to the extent that the presence of isocupressic acids cannot be detected by conventional techniques and/or has no effect on the properties of the composition).

The composition is obtainable, and is preferably obtained, from pine needles. Pine needles are preferably from species of pine other than *Pinus ponderosa*. Pine species include *Pinus albicaulis, Pinus aristata, Pinus attenuata, Pinus balfouriana, Pinus banksiana, Pinus bungeana, Pinus cembra, Pinus cembroides, Pinus clausa, Pinus contorta, Pinus coulteri, Pinus densiflora, Pinus echinata, Pinus edulis, Pinus elliottii, Pinus engelmannii, Pinus flexilis, Pinus glabra, Pinus heldreichii, Pinus jeffreyi, Pinus lambertiana, Pinus longaeva, Pinus massoniana, Pinus monophylla, Pinus monticola, Pinus mugo, Pinus muricata, Pinus nigra, Pinus palustris, Pinus parviflora, Pinus pungens, Pinus quadrifolia, Pinus radiata, Pinus resinosa, Pinus rigida, Pinus sabiniana, Pinus serotina, Pinus strobiformis, Pinus strobus, Pinus sylvestris, Pinus tabulaeformis, Pinus taeda, Pinus thunbergiana, Pinus torreyana, Pinus virginiana, Pinus yuannensis* and *Pinus washoensis*. Preferably, the composition is from *Pinus massoniana*, *Pinus tabulaeformis* or *Pinus yuannensis*, more preferably, the material is from *Pinus massoniana*. The composition preferably comprises one or more organic compounds, more preferably two or more organic compounds. Organic compounds are compounds that comprise carbon, hydrogen and oxygen atoms and optionally other atoms such as nitrogen, phosphorus and sulphur.

The composition preferably comprises at least 2 components A and B, wherein A is a compound that is obtainable from a mixture of A and B by elution from a silica column using 100 % methanol as eluent and B is a compound obtainable from the same silica column using methanol/water mixtures (5-40 % by volume) in a series of subsequent elutions. A is preferably selected from the group consisting of phytosterol, polyphenols, bioflavonoids, tannins, organic acids and their complexes and at least one compound selected from the group consisting of amino acids, peptides, proteins, quercetin, terpenoids, flavonol glycosides, biflavones, proanthocyanidins, polyprenols, lignans and minerals. The composition may comprise one or more compounds A and one or more compounds B. Preferably, the composition comprises A (or total A compounds where more than one A compound is present) in an amount of from 5 to 60 wt %, preferably 10 to 50 wt%, most preferably 15 to 40 wt%, and the composition comprises B (or total B compounds where more than one B compound is present) in an amount of from 1 to 15 wt %, preferably 2 to 12wt %, most preferably 3 to 10 wt%, based on the weight of the composition.

Therefore, in one embodiment, the composition of the invention comprises at least one compound selected from the group consisting of phytosterol, polyphenols, bioflavonoids, tannins, organic acids and their complexes and at least one compound selected from the group consisting of amino acids, peptides, proteins, quercetin, terpenoids, flavonol glycosides, biflavones, proanthocyanidins, polyprenols, lignans and minerals.

Compositions of the invention may be used in a foodstuff (for example a dairy based food product), food supplement or pharmaceutical composition. These products provide a convenient form in which to deliver the composition.

Compositions of the invention may comprise an antioxidant in an amount effective to increase the stability of the composition with respect to oxidation and optionally colouring agents and/or preservatives.

A preferred composition according to the invention is a foodstuff. Foodstuffs include liquids (e.g, beverages) and solids. Suitably, foodstuffs will be packaged and labelled as foodstuffs. Conventional foodstuffs may incorporate the composition of the invention in a suitable amount.

Pharmaceutical compositions may, for example, be in the form of tablets, pills, capsules, caplets, multiparticulates including: granules, beads, pellets and micro-encapsulated particles; powders, elixirs, syrups, suspensions and solutions. Pharmaceutical compositions will comprise a pharmaceutically acceptable diluent or carrier. Pharmaceutical compositions are preferably adapted for administration parenterally (e.g., orally). Orally administrable compositions may be in solid or liquid form and may take the form of tablets, powders, suspensions and syrups. Optionally, the compositions comprise one or more flavouring and/or colouring agents. Pharmaceutically acceptable carriers suitable for use in such compositions are well known in the art of pharmacy. The pharmaceutical compositions of the invention may contain 0.1-99% by weight of the composition of the invention. Pharmaceutical compositions of the invention are generally prepared in unit dosage form.

Further examples of product forms that comprise the composition are food supplements, such as in the form of a soft gel or a hard capsule, preferably comprising an encapsulating material selected from the group consisting of gelatin, starch, modified starch, starch derivatives such as glucose, sucrose, lactose and fructose. The encapsulating material may optionally contain cross-linking or

polymerizing agents, stabilizers, antioxidants, light absorbing agents for protecting light-sensitive fills, preservatives and the like. Preferably, the unit dosage of the composition of the invention in the food supplements is from 1mg to 1000mg (more preferably from 100mg to 750mg).

The foodstuff of the invention is preferably a dairy based food product. The dairy

10wt%, more preferably from 0.05wt% to 5wt%, even more preferably from 0.1wt% to 2wt%, based on the total weight of the dairy based food product and based on the dry weight of the composition. Dairy based food products include edible products comprising one or more proteins, fats and/or sugars derived from milk. Milk proteins include, for example, casein and milk sugars include, for example, lactose. Preferably, the dairy based food product comprises milk proteins in an amount of at least 0.01% by weight, more preferably 0.1% by weight, even more preferably 1% by weight, based on the weight of the dairy based food product.

Dairy based food products of the invention preferably have a water content of from 0.5 to 99.5 wt %, preferably 20 to 90 wt%, most preferably 30 to 85 wt%.

Dairy based food products are typically oil in water (O/W) emulsions, bicontinuous emulsions or duplex W/O/W (water in oil in water) emulsions.

Certain dairy based food products of the invention comprise a fat phase. The fat phase preferably displays a solid fat content (measured by NMR on a non-stabilised fat) at 5°C (=N5) of >10, preferably >20, and at 35°C (=N35) of <20, preferably <10, most preferably less than 5. Methods for determining solid fat content by NMR on non-stabilised fat are well known to those skilled in the art

and include the method described in Fette, Seifen, Anstrichmittel, 80 (1978), 180-186. Non-stabilised means that the N-value is measured after first melting the fat above 80° C, whereupon the melt is cooled to 0° C and kept at 0° C for 30 minutes, then the fat is heated to the measurement temperature and kept at that temperature for 30 minutes, whereupon the N-value is measured.

Examples of dairy based food products of the invention are cream, milk, water continuous or bicontinuous spreads, confectionery or sweet spreads, chocolate, ice cream, confectionery fillings or toppings, bakery fillings or toppings, yoghurt, including drinkable yoghurt, curd cheese, milk shake, slimming drinks, cheese and cheese spreads.

Preferably, the dairy based food products of the invention are essentially free of trans fatty acids (which are carboxylic acids containing from 12 to 24 carbon atoms and having one carbon-carbon double bond) i.e, they contain trans fatty acids in an amount of less than 1% by weight, preferably less than 0.5% by weight, more preferably less than 0.1% by weight, such as less than 0.05% or less than 0.01% by weight.

The dairy based food product of the invention preferably has one or more of the following properties compared to a corresponding product that does not contain the composition: improved hardness, improved texture, improved aeration, improved spreadability, improved oral properties, improved mouthfeel, improved flavour, better colour, improved viscosity, improved whipping properties, improved ease of processing. The properties are improved compared to an otherwise identical food product that does not contain the material. Preferred properties that are improved according to the invention are oral properties and/or

visual appearance, in particular increased similarity to butter in terms of oral properties and/or visual appearance.

The following non-limiting examples illustrate the invention and do not limit its scope in any way. In the examples and throughout this specification, all percentages, parts and ratios are by weight unless indicated otherwise.

Examples

Reference is made in the examples to Figure 1.

Figure 1 shows the dose dependent contraction of rat aorta caused by phenylephrine and the inhibition of this effect by a pine needle extract of the invention.

Example 1

Pine needle extraction

100 g pine needles from *Pinus massoniana* (isocupressic acids (ICA) content 0.33wt%) were cleaned with water, cut into small pieces (3~4 cm) and put in a flask. 500 g hexane was added to the flask and heated under stirring to reflux (~ 60°C) for about 3 to 5h. The resulting pine needle solution was filtered through a Büchner funnel and the hexane removed using a rotary evaporator. The crude extract contains 8wt% of compounds of the isocupressic acid family. This extract was not used for further experiments.

The residue which was left after treatment with hexane was transferred to a flask and 500 ml demineralised water was added. The mixture was stirred at 100°C for about 3-5h. Then the extract was filtered through a Büchner funnel and concentrated to 150 ml. To this extract 12.5g resin (Dowex Marathon A, Polysep Industrial Consultants) was added; the temperature was maintained at 50°C for 3h. After filtration through a Büchner funnel to remove the resin, the solution was dried in a rotary evaporator to produce the pine needle powder (ICA content 0.003wt%).

Example 2

Thoracic aortas were obtained from spontaneous hypertensive rats (SHR). The thoracic aorta is cut into rings of 4 to 6 mm in length and each ring is connected to a tension transducer in a thermostatically controlled and oxygenated organ bath containing modified Krebs-Henselheit buffer. The contractions of rings of aorta are recorded continuously under isotonic conditions. After equilibrating the tissues, a single dose of 1μM phenylephrine was given to sensitise the tissue, followed by washout. Hereafter, two cumulative dose response curves of phenylephrine were generated. The first dose response curve was obtained in the absence of an extract and served as a control curve. After thorough washing (7 times) the tissues were incubated with the pine needle extract for 1 hour. Following this incubation period, a second dose response curve was obtained in the presence of a concentrated form of the extract. The data were analysed taking the maximal response of the reference curve as a control.

Figure 1 shows that phenylephrine causes a dose dependent contraction of rat aorta (the upper curve in the Figure). After incubation with pine needle extract of

Example 1, the contraction of rat aorta by phenylephrine is clearly inhibited (the lower curve in the Figure).

Claims

1. Composition which is obtainable as an extract from pine needles, having therapeutic activity and comprising isocupressic acid compounds in an amount of less than 0.01 wt%.

2. Composition according to Claim 1, which contains isocupressic acid compounds in an amount of less than 0.005 wt%, preferably less than 0.003 wt%.

3. Composition according to Claim 1 or Claim 2 comprising at least 2 components A and B, wherein A is a compound that is obtainable from a mixture of A and B by elution from a silica column using 100 % methanol as eluent and B is a compound obtainable from the same silica column using methanol/water mixtures (5-40 % by volume) in a series of subsequent elutions.

4. Composition according to Claim 3, wherein compound A is selected from the group consisting of phytosterol, polyphenols, bioflavonoids, tannins, organic acids and their complexes.

5. Composition according to Claim 3 or Claim 4, wherein compound B is selected from the group consisting of amino acids, peptides, proteins, quercetin, terpenoids, flavonol glycosides, biflavones, proanthocyanidins, polyprenols, lignans and minerals.

6. Composition as claimed in Claim 1 or Claim 2 which comprises a compound A selected from the group consisting of phytosterol, polyphenols, bioflavonoids, tannins, organic acids and their complexes and a compound B selected from the group consisting of amino acids, peptides, proteins, quercetin, terpenoids, flavonol glycosides, biflavones, proanthocyanidins, polyprenols, lignans and minerals.

7. Composition according to any one of Claims 3 to 6, wherein A is present in an amount of from 5 to 60 wt %, preferably 10 to 50 wt%, most preferably 15 to 40 wt%, and B is present in an amount of from 1 to 15 wt %, preferably 2 to 12wt %, most preferably 3 to 10 wt%, based on the weight of the composition.

8. Foodstuff, food supplement or pharmaceutical composition comprising a composition of any one of Claims 1 to 7.

9. Dairy based food product comprising a composition as claimed in any one of Claims 1 to 7.

10. Dairy based food product according to Claim 9 having a water content of from 0.5 to 99.5 wt %, preferably 20 to 90 wt%, most preferably 30 to 85 wt%.

11. Dairy based food product according to Claim 9 or Claim 10 which is an oil in water (O/W) emulsion, a bicontinuous emulsion or a duplex W/O/W emulsion.

12. Dairy based food product according to Claim 9, which is a cream, milk, water continuous or bicontinuous spread, confectionery or sweet spread, chocolate, ice cream, confectionery filling or topping, bakery filling or topping, yoghurt, drinkable yoghurt, curd cheese, milk shake, slimming drink, cheese or cheese spread.

13. Dairy based food product according to any one of Claims 9 to 12, comprising a fat phase that displays a solid fat content (measured by NMR on a non-stabilised fat) at 5°C (=N5) of >10, preferably >20, and at 35°C (=N35) of <20, preferably <10, most preferably less than 5.

14. Dairy based food product as claimed in any one of Claims 9 to 13 which is essentially free of trans fatty acids.

15. Dairy based food product according to any one of Claims 9 to 14 which comprises from 0.05wt% to 10wt% of the composition of any one of Claims 1 to 7.

16. Dairy based food product according to any one of Claims 9 to 15 which has one or more of the following properties compared to a corresponding product that does not contain the composition: improved hardness, improved texture, improved aeration, improved spreadability, improved oral properties, improved mouthfeel, improved flavour, better colour, improved viscosity, improved whipping properties and improved ease of processing.

17. Composition as claimed in any one of Claims 1 to 7, foodstuff, food supplement or pharmaceutical composition as claimed in Claim 8 or

dairy based food product as claimed in any one of Claims 9 to 15 for use to lower blood pressure in mammals, particularly in humans.

18. The use of a composition as claimed in any one of Claims 1 to 7, a foodstuff, food supplement or pharmaceutical composition as claimed in Claim 8 or a dairy based food product as claimed in any one of Claims 9 to 15 for the manufacture of a medicament for use to lower blood pressure in mammals, particularly in humans.

19. A method of lowering blood pressure in a mammal, particularly a human, which comprises providing the mammal with an effective amount of a composition as claimed in any one of Claims 1 to 7, a foodstuff, food supplement or pharmaceutical composition as claimed in Claim 8 or a dairy based food product as claimed in any one of Claims 9 to 15.

20. A method of improving one or more properties of a food product selected from hardness, texture, aeration, spreadability, oral properties, mouthfeel, flavour, colour, viscosity, ease of processing and health properties, which comprises incorporating into the food product a composition comprising one or more organic compounds, said composition being obtainable as an extract from pine needles.

21. Use of a material comprising one or more organic compounds, said material being obtainable as an extract from pine needles, for improving one or more properties of a food product selected from hardness, texture, aeration, spreadability, oral properties, mouthfeel, flavour, colour, viscosity, ease of processing and health properties.

22. Method as claimed in Claim 19 or Claim 20, or use as claimed in Claim 18 or Claim 21, wherein the composition has therapeutic activity and comprises isocupressic acid compounds in an amount of less than 0.01 wt%.

23. Process for producing the composition of any one of Claims 1 to 7, which comprises the following steps:

a. treating pine needles with a solvent selected from water, organic solvents and mixtures thereof, to form a first extract;
b. removing isocupressic acid compounds from the first extract by treatment with an ion exchange resin; and
c. optionally, filtering and concentrating the treated extract to obtain the composition as a powder or a concentrate.

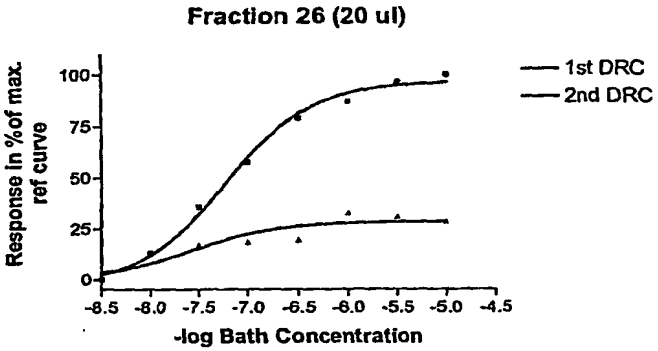
24. Process as claimed in Claim 23, wherein prior to step (a), the pine needles are pretreated with a non-polar solvent.

Abstract

Compositions

A composition which is obtainable as an extract from pine needles, having therapeutic activity and comprising isocupressic acid compounds in an amount of less than 0.01 wt%, can be used in foodstuffs, pharmaceutical compositions and food supplements.

Figure 1.



	1st DRC	2nd DRC
BOTTOM	-2.550	0.7572
TOP	96.67	28.58
LOGEC50	-7.225	-7.539
EC50	5.9620e-008	2.8910e-008

Figure 1